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Original

Methane Emission by the Termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) I.

Effects of Termite Caste, Population Size and Volume of Test Containers

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Abstract——Methane emission by termites, *Coptotermes formosanus* Shiraki was investigated in relation to termite caste, population size and volume of test containers. Workers constantly emitted methane within the first 72 hr, whereas emission rates of soldiers gradually decreased after 6 hr test duration. Workers and soldiers showed maximum emission rates of 0.75 and 0.019 nmol/termite/hour, respectively, and no significant differences were observed regardless of test container volume when five or more individuals were served for measuring methane emission by workers. In addition, no conspicuous relationships between the volume of test containers and methane emission of workers were observed. Those findings suggested that physiological changes of termites through the year might be major factors which affected the methane emission by termites.

Keywords: Methane emission, global warming, *Coptotermes formosanus*, termite caste, population size, volume of test container.

1. Introduction

Global warming has been becoming a great public concern in recent years as the five global-average warmest years in the past century occurred in the 1980's[1]. Large quantities of carbon dioxide and other heat-trapping gases are released to the atmosphere through modern industrialized life activities such as burning of fossil fuels and deforestating.

Methane, which is comprised in the important trace gases accounting for the "greenhouse" effect, is present at concentrations of 1.6–1.7 ppm in the atmosphere. The global concentration of methane is steadly increasing at a rate of approximately 2% per annum[2,3]. Methane is emitted into the atmosphere primarily from decomposition of organic materials in wetlands and rice paddy fields, and also produced by cattle and termites as the by-product of digestion.

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As the scientific records indicate, termites are distributed on approximately two-thirds of the land area of the earth. Although only 10% of the total termite species are capable of attacking wood, they are considered to play a significant role in accelerating the pace of recycles of soil nutrients by decomposition of cellulosic materials such as dead bodies and leaves of plants in the warm regions. Termites thus are great contributors to the ecosystem conversion.

Since Breznak evidenced the methane emission by three species of termites in 1975[4], the role of termites in the global methane production has been investigated by some scientists[5–11]. It is boldly estimated that termites are responsible for less than 5%[5] to 40%[9] of the global methane emissions, which large uncertainties would not allow accurate estimation. As most of former reports were mainly provided by geophysicists except for a couple of field experiments by entomologists[10,11], detailed mechanism of methane production by termites remained unsolved.

More recently Messer *et al.* investigated the role of microbiota in methane emission by termites with the aids of various chemical treatments, and concluded that the ptotozoa, *Trichomitopsis,* produced most of the methane in the hindgut ecosystem of *Zootermopsis angusticollis*[12].

In this paper, methane emission by *Coptotermes formosanus* Shiraki is discussed with regard to termite caste, population size and volume of test containers as the first step in order to understand interactions among various microorganisms involved in methane emission.

2. Materials and Methods

2.1 Termites

Termites employed in a series of present experiments were externally undifferentiated mature larvae (=workers) of *Coptotermes formosanus* Shiraki from a laboratory colony maintained at 28±2°C and over 85% R.H. with wood pieces of Akamatsu (*Pinus densiflora* Sieb. et Zucc).

2.2 Quantification of methane emission

2.2.1 Test containers

Glass vials, Erlenmeyer flasks and incubation box were used to cultivate termites for quantifying the methane emission. Those containers have an available volumes of 51.00 ml, 130.27 ml on the average and 11.915 *l*, respectively.

2.2.2 Test procedure

(a) Glass vial test

Workers or soldiers (1, 2, 5, 10 or 20 individuals) of *C. formosanus* and a damp filter paper were placed in glass vials with a silicon rubber cap, and incubated at 26±2°C in the dark. After the desired incubation periods (6, 24, 48, 72 and 144 hr), a 1 ml gas sample was taken by a precision syringe after gentle rotation of vials to make even methane

concentration. The sample was then served for GC analysis. Three replicates were prepared for each incubation period.

(b) Erlenmeyer flask test

Workers (15, 30, 50 or 75 individuals) of *C. formosanus* were similarly incubated in Erlenmeyer flasks for 24, 48 and 72 hr. After incubation, a sample of 1 ml gas was taken by a precision syringe and was served for GC analysis. Five replicates were prepared for each incubation period.

(c) Incubation box test

Workers (30, 150 or 300 individuals) of *C. formosanus* were put into an acrylic test cylinder (80 mm in diameter and 60 mm in height) with the plaster bottom. An assembled container was then set on damp cotton pads so that the temites could take water through the plaster bottom during the test duration, and the test set was placed in the incubation box which was already regulated at 30°C in the dark as shown in Fig. 1. After the desired periods of incubation (24, 48 and 72 hr), the air in the box was agitated by small fan which was attached on the side wall of the box to make the methane concentration uniform prior to taking samples. A 1 ml sample was taken by a precision syringe from the rubber cap and served for GC analysis. Three replicates were prepared for each incubation period.

2.2.3 GC analysis

Methane amount in the samples was measured using a Shimadzu GC-15A gas chromatograph fitted with a flame ionization detector (FID); a glass column (1.6 m long, 3 mm i.d.) packed with 80/100 Porapak Q; flow rate (N_2) 25 ml/min; column temperature 32°C; detector temperature 50°C. An external methane standard (99.7%, GL Sciences Co.

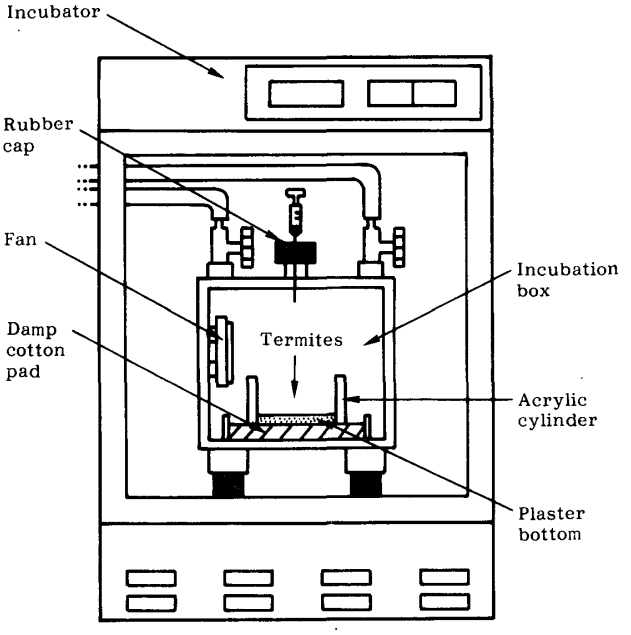

Fig. 1. Incubation box.

Ltd.) was used, and the methane concentrations were calculated as the total methane emission (nmol/termite) and the methane emmision rates (nmol/termite/hour).

3. Results and Discussion

3.1 Methane emission by workers

Figure 2 shows the total methane emission by workers of *C. formosanus*. Workers constantly emitted methane within the first 72 hr in any case, and the total methane emission reached 20–50 nmol/termite for glass vial test (Fig. 2-A), 20–25 nmol/termite for Erlenmeyer flask test (Fig. 2-B) and 45–55 nmol/termite for incubation box test (Fig. 2-C) at 72 hr.

After that time methane emission by workers appeared to slightly decrease possibly because of the effect of the artificial culturing in the small chambers on the health conditions of workers tested (Fig. 2-A). This tendency can be recognized more clearly when the data were converted to methane emission rates per hour (Tables 1–3). As indicated in Table 1, methane emission rates from 72 hr to 144 hr dropped to approximately 80% in comparison

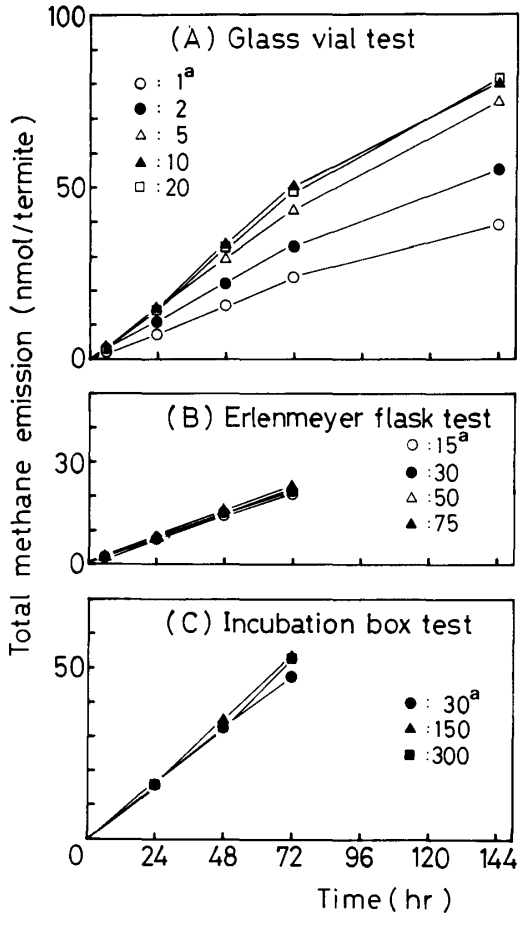

Fig. 2. Total methane emission by workers of *Coptotermes formosanus* Shiraki. (A) : Glass vial test, (B) : Erlenmeyer flask test, (C) : Incubation box test. a : Population size.

Table 1. Methane emission rates of workers of *Coptotermes formosanus* Shiraki in glass vial tests.

Number of individuals	Methane emission rates (nmol/termite/hour)[a]				
	Incubation time (hr)				
	0–6	6–24	24–48	48–72	72–144
1	0.326	0.297	0.331	0.330	0.273
2	0.440	0.463	0.469	0.464	0.386
5	0.587	0.652	0.613	0.606	0.522
10	0.633	0.630	0.698	0.699	0.558
20	0.520	0.590	0.677	0.679	0.564

a: Mean values of three replicates.

Table 2. Methane emission rates of workers of *Coptotermes formosanus* Shiraki in Erlenmeyer flask tests.

Number of individuals	Methane emission rates (nmol/termite/hour)[a]			
	Incubation time (hr)			
	0–6	0–24	24–48	48–72
15	0.317	0.330	0.292	0.254
30	0.320	0.326	0.318	0.274
50	0.315	0.319	0.321	0.285
75	0.391	0.356	0.353	0.268

a: Meanvalues of five replicates.

Table 3. Methane emission rates of workers of *Coptotermes formosanus* Shiraki in incubation box tests.

Number of individuals	Methane emission rates (nmol/termite/hour)[a]		
	Incubation time (hr)		
	0–24	24–48	48–72
30	0.645	0.674	0.661
150	0.661	0.735	0.756
300	0.653	0.673	0.739

a: Mean values of three replicates.

with values before 72 hr.

Maximum emission rates ranged from approximately 0.39 nmol/termite/hour in Erlenmeyer flask test (Table 2) to 0.70–0.75 nmol/termite/hour in both glass vial test and incubation box test (Tables 1 and 3). A few of research works on the methane emission of Rhinotermitidae termites reported the following emission rates: 1.3 mg/kg termite/hour (Unit) for *Coptotermes acinaciformus* (Frogatt)[5]; 1.1 U for *Coptotermes lacteus* (Frogatt)[5]; 0.06 U

for *C. formosanus*[5] ; 0.4 U for *Reticulitermes flavipes* (Kollar)[4] ; 0.6 U for *Reticulitermes tibialis* (Banks)[9]. Converting the present data into Unit, the maximum methane emission rate (0.75 nmol/termite/hour) was equivalent to 3.4 U, and this was over fifty times higher than the above cited value for *C. formosanus* possibly due to many experimantal and physiological factors such as test season, activity of colony and instars of termite individuals *etc.*

Although maximum methane emission rates was fairly varied with test volume as described above, noticeable relationships could not be seen between volume of test containers and methane emission by workers. Glass vial test and incubation box test were conducted in March, 1992 at the same time, and Erlenmeyer flask test was independently conducted in August, 1992. Despite of the constant temperature and humidity in termite culturing room all through the year, it is well recognized that the foraging activities of workers fluctuate seasonally to some extent. Therefore, significantly lower methane emission in Erlenmeyer flask test might be caused by seasonal changes of physiological conditions of termites.

Significant effects of population size were observed only in glass vial test (Fig. 2-A). In the cases of a single and two workers, termite totally emitted approximately a half and two-thirds of methane in the cases of five and more individuals in 72 hr test duration, respectively. Consequently, it was evidenced that over five individuals were needed to quantify the methane emission by workers of *C. formosanus*. In addition, incubation periods should be shorter than 72 hr, and the various tests should be conducted at the same time to facilitate comparison of the test results.

3.2 Methane emission by soldiers

Methane emission by soldiers of *C. formosanus* was measured using only glass vials. Results are summarized in Table 4. Emission rates of soldiers showed maximum values within the first 6 hr and then decreased gradually with time when two or more individuals were served for measurements. Methane emission from a single soldier could not be

Table 4. Methane emission rates of soldiers of *Coptotermes formosanus* Shiraki in glass vial tests.

Number of individuals	Methane emission rates (nmol/termite/hour)[a]				
	Incubation time (hr)				
	0–6	6–24	24–48	48–72	72–144
1	−0.017	−0.002	0.002	−0.002	0.000
2	0.019	0.010	0.009	0.007	0.006
5	0.017	0.011	0.010	0.007	0.006
10	0.016	0.018	0.014	0.011	0.010
20	0.012	0.010	0.009	0.007	0.006

a : Mean values of three replicates.

detected. As soldiers can not ingest foods by themselves, decreasing emission rates with time may be caused by lack of nutritions.

Maximum emission rates of soldiers did not go up to one-thirtieth of those of workers (Table 1). Methane emission by lower termites is believed to be dependent on methanogenic bacteria associated with symbiotic protozoa in the hindgut[13]. Workers and soldiers of *C. formosanus* possess the three protozoan species in their hindgut[14]. Lai *et al.* reported that soldiers of *C. formosanus* possessed only approximately one-eighth of total number of protozoa as compared with those of workers[15]. It, thus, seems that the number of protozoa in the hindgut of *C. formosanus* is closely related to the methane emission as already shown in *Zootermopsis angusticollis*[12].

However, symbiotic relationships between protozoa and methanogenic bacteria in *C. formosanus* still remain unsolved in terms of methane emission. Microscopic observations and physiological studies combined with the selective defaunation techniques of protozoa will be further needed to understand roles of each protozoa species.

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